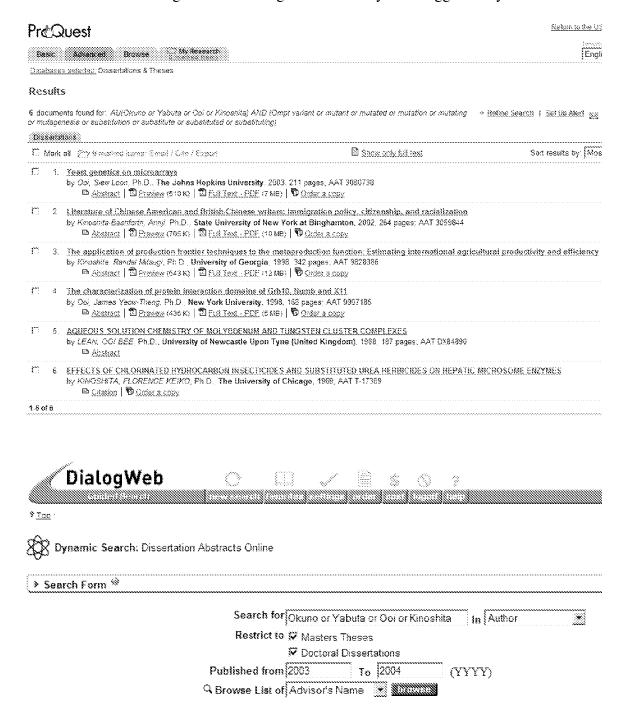
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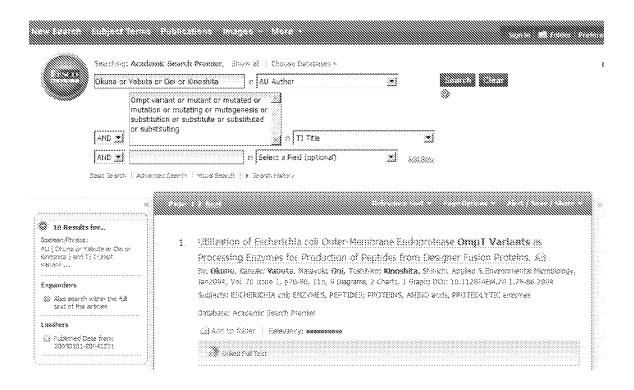
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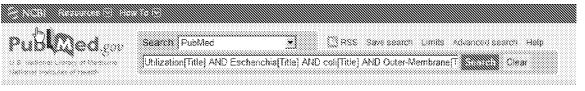
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Appl Environ Microbiol, 2004 Jan;70(1):78-86

Utilization of Escherichia coli outer-membrane endoprotease OmpT variants as processing enzymes for production of peptides from designer fusion proteins.

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Abstract

Escherichia coli outer-membrane endoprotease OmpT has suitable properties for processing fusion proteins to produce peptides and proteins. However, utilization of this protease for such production has been restricted due to its generally low cleavage efficiency at Arg (or Lys)-Xaa, where Xaa is a nonbasic N-terminal amino acid of a target polypeptide. The objective of this study was to generate a specific and efficient OmpT protease and to utilize it as a processing enzyme for producing various peptides and proteins by converting its substrate specificity. Since OmpT AspiS7) is proposed to interact with the P1 amino acid of its substrates. OmpT variants with variations at AspiS7; were constructed by replacing this amino acid with 19 natural amino acids to after the cleavage specificity at Arg (P1)-Xaa (P1). The variant OmpT that had a methionine at this position, but not the wild-type OmpT, efficiently cleaved a fusion protein containing the amino acid sequence -Arg-Arg-Arg-Ala-Arg downward arrow motifin, in which motifin is a model peptide with a phenylaianine at the N terminus. The OmpT variants with leurine and fusicinine at position 97 were useful in releasing human adrenocorticotropic hormone (1-24) (serine at the N terminus) and human calcitonin precursor (cysteine at the N terminus), respectively, from fusion proteins. Motifin was produced by this method and was purified up to 99 6% by two chromatographic steps; the yield was 150 mg/liter of culture. Our novel method in which the OmpT variants are used could be employed for production of various peptides and proteins.

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OWN - NLM

STAT- MEDLINE

DA - 20040108

DCOM- 20040408

LR - 20100617

IS - 0099-2240 (Print)

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VI - 70

TP - 1

DP - 2004 Jan

TI - Utilization of Escherichia coli outer-membrane endoprotease OmpT variants as

 $\,$ processing enzymes for production of peptides from designer fusion proteins.

PG - 76-86

AB - Escherichia coli outer-membrane endoprotease OmpT has suitable properties for

processing fusion proteins to produce peptides and proteins. However, utilization $% \left(1\right) =\left(1\right) +\left(1\right) +$

of this protease for such production has been restricted due to its generally low

cleavage efficiency at Arg (or Lys)-Xaa, where Xaa is a nonbasic N-terminal amino $\,$

acid of a target polypeptide. The objective of this study was to generate a $% \left(1\right) =\left(1\right) +\left(1\right)$

specific and efficient OmpT protease and to utilize it as a processing enzyme for

producing various peptides and proteins by converting its substrate specificity.

Since OmpT Asp(97) is proposed to interact with the P1' amino acid of its

substrates, OmpT variants with variations at Asp(97) were constructed by

replacing this amino acid with 19 natural amino acids to alter the cleavage

specificity at Arg (Pl)-Xaa (Pl'). The variant \mbox{OmpT} that had a methionine at this

position, but not the wild-type $\ensuremath{\mathsf{OmpT}}$, efficiently cleaved a fusion protein

containing the amino acid sequence $-\mathrm{Arg-Arg-Ala-Arg}$ downward arrow motilin,

in which motilin is a model peptide with a phenylalanine at the N terminus. The $\,$

 \mbox{OmpT} variants with leucine and histidine at position 97 were useful in releasing

human adrenocorticotropic hormone (1-24) (serine at the N terminus) and human $\,$

proteins. Motilin was produced by this method and was purified up to 99.0% by two

chromatographic steps; the yield was 160 mg/liter of culture. Our novel method in

which the OmpT variants are used could be employed for production of various $\ensuremath{\mathsf{C}}$

peptides and proteins.

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RN - 0 (Bacterial Outer Membrane Proteins)

RN - 0 (Escherichia coli Proteins)

RN - 0 (Peptides)

RN - 0 (Porins)

RN - 0 (Recombinant Fusion Proteins)

RN - 0 (ompT protein, E coli)

RN - 52906-92-0 (Motilin)

RN - EC 3.4.- (Peptide Hydrolases)

RN - EC 3.4.21.- (Serine Endopeptidases)

SB - IM

MH - Amino Acid Sequence

MH - Bacterial Outer Membrane Proteins

- MH Escherichia coli/*enzymology/genetics
- MH Escherichia coli Proteins
- MH - *Genetic Variation
- MH - Humans
- MH Inclusion Bodies/metabolism
- MH Molecular Sequence Data
- MH Motilin/genetics/metabolism
- MH Mutation
- MH Peptide Hydrolases
- MH Peptides/*metabolism
- MH Porins/genetics/*metabolism
- MH Recombinant Fusion Proteins
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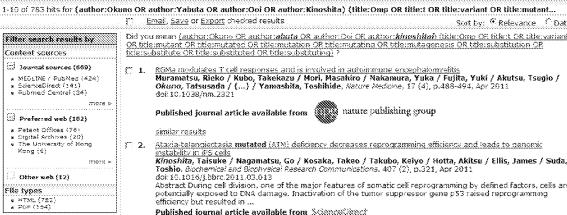


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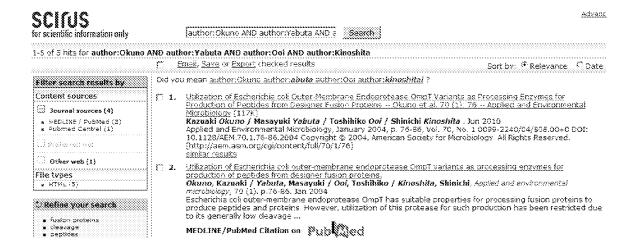
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Abstract During cell division, one of the major features of somatic cell reprogramming by defined factors, cells are potentially exposed to DNA damage. Inactivation of the tumor suppressor gene p53 raised reprogramming efficiency but resulted in ...

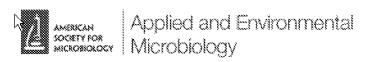
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Utilization of Escherichia coli Outer-Membrane Endoprotease OmpT Variants as Processing Enzymes for Production of Peptides from Designer Fusion Proteins

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